

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
16 January 2003 (16.01.2003)

PCT

(10) International Publication Number  
**WO 03/004495 A1**

(51) International Patent Classification<sup>7</sup>: **C07D 471/04**,  
491/052, 495/04, A61K 31/4745, 31/4162, A61P 3/10,  
11/06, 17/06, 19/02, 37/02

(21) International Application Number: **PCT/SE02/01309**

(22) International Filing Date: **2 July 2002 (02.07.2002)**

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:  
0102404-1 **4 July 2001 (04.07.2001)** **SE**

(71) Applicant (*for all designated States except US*): **ACTIVE BIOTECH AB** [SE/SE]; Box 724, S-220 07 Lund (SE).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **BJÖRK, Per, Axel** [SE/SE]; Sigrïdsgatan 31, S-256 56 Helsingborg (SE). **FEX, Tomas** [SE/SE]; Harpasset 1, S-222 52 Lund (SE). **PETTERSSON, Lars, Olof, Göran** [SE/SE]; Näktergalsvägen 33, S-247 36 Södra Sandby (SE). **SØRENSEN, Poul** [DK/DK]; Peder Skrams Gade 27 4th, DK-1054 København K (DK). **DA GRACA THRIGE, Dorthé** [DK/DK]; Langagervej 28, DK-2500 Valby (DK).

(74) Agent: **AWAPATENT AB**; Box 5117, S-200 71 Malmö (SE).

(81) Designated States (*national*): AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: **NOVEL IMMUNOMODULATING COMPOUNDS**

(57) Abstract: The present invention relates to a novel heterocyclic compound, a pharmaceutical composition comprising said compound, a method and use of said compound for clinical treatment of medical conditions which may benefit from immunomodulation, e.g. rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis. More particularly the present invention relates to novel heterocyclic compounds, which are CD80 antagonists capable of inhibiting the interactions between CD80 and CD28.



**WO 03/004495 A1**

## NOVEL IMMUNOMODULATING COMPOUNDS

### Field of the invention

The present invention relates to immunomodulating compounds, pharmaceutical compositions comprising said compounds, use of said compound as well as a method for  
5 treatment of medical conditions which benefit from immunomodulation, wherein said compounds are administered.

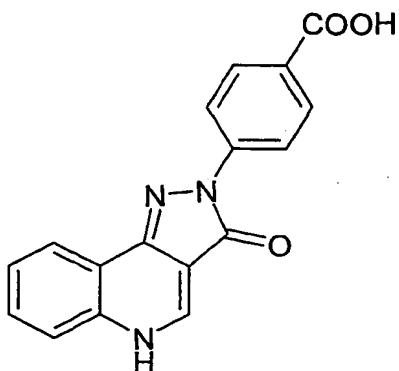
### Background of the invention

The immune system possesses the ability to control the homeostasis between the activation and inactivation  
10 of lymphocytes through various regulatory mechanisms during and after an immune response. Among these are mechanisms that specifically inhibit and/or turn off an immune response. Thus, when an antigen is presented by MHC molecules to the T-cell receptor, the T-cells become properly  
15 activated only in the presence of additional co-stimulatory signals. In the absence of accessory signals there is no lymphocyte activation and either a state of functional inactivation termed anergy or tolerance is induced, or the T-cell is specifically deleted by apoptosis.

20 One such co-stimulatory signal involves interaction of CD80 on specialised antigen-presenting cells with CD28 on T-cells, which has been demonstrated to be essential for full T-cell activation. (Lenschow et al. (1996) Annu. Rev. Immunol., 14, 233-258).

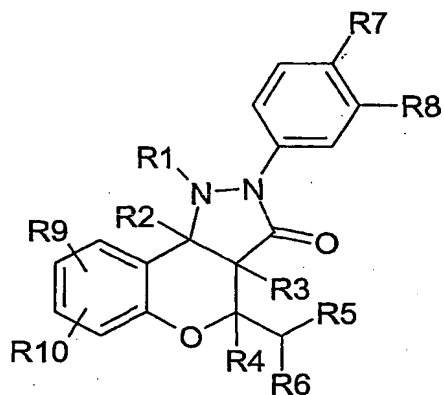
### 25 Prior art

In US 4,312,870 compound A is disclosed as one of several psychoactive compounds but without any biological data. Some related compounds are described by A. Carotti in Bioorganic & Medicinal Chemistry 6 (1998) 389 - 399,  
30 and from data related to these compounds it is obvious that the carboxylic acid substituent greatly diminishes biologic activity measured as affinity for the CNS benzodiazepine receptor.

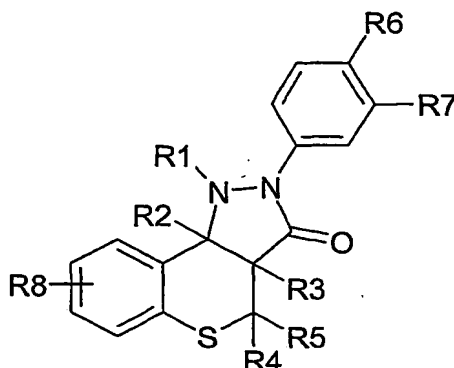


A

EP 0354693A1 (Boots) discloses immunomodulatory compounds of general structure B but does not include structures wherein R7 and/or R8 are COOH or contain a COOH group.

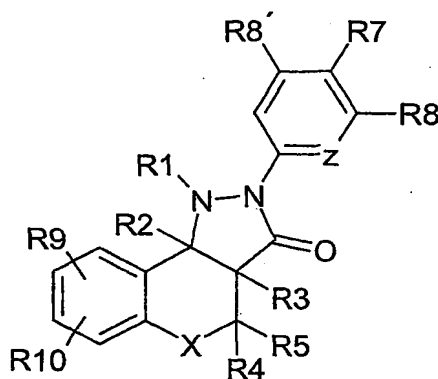


3



C

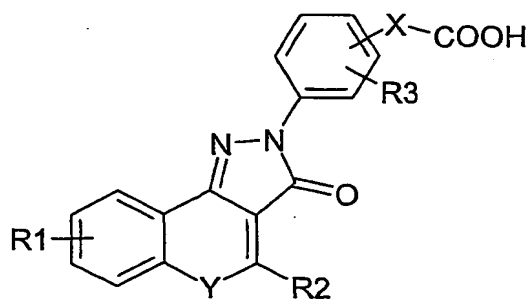
Also, WO9111448 (Boots) discloses immunomodulatory compounds of general structure D but here are no structures wherein R7 and/or R8 and R8' are COOH or contain a  
5 COOH group.



D

### Summary of the invention

The present invention relates in a first aspect to a novel compound having the general formula (I)



(I)

wherein X represents a bond or a group selected from substituted or unsubstituted C<sub>1-3</sub>-alkyl, NH-C(O)-C<sub>1-3</sub>-alkyl, NH-C(O)-CH<sub>2</sub>-O-CH<sub>2</sub> or C(O)-NH-(amino acid residue); Y represents NR<sub>4</sub>, O or S;

- 5 R<sub>1</sub> represents H, halo, CF<sub>3</sub>, lower alkyl or lower alkoxy; R<sub>2</sub> and R<sub>4</sub> represents independently H or lower alkyl; and R<sub>3</sub> represents H, halo, lower alkyl or lower alkoxy, wherein halo is F, Cl or Br; wherein lower alkyl represents saturated or unsaturated, 10 straight, branched or cyclic alkyl groups having 1-6 carbon atoms; and wherein lower alkoxy represents saturated or unsaturated, straight, branched or cyclic alkoxy groups having 1-6 carbon atoms, 15 with the proviso that R<sub>2</sub> is not H, when X is a bond and Y is NH and R<sub>3</sub> is H, or pharmaceutically acceptable salts thereof.

In one preferred embodiment of the invention the compound X is a bond and in another preferred embodiment 20 Y is NH.

- In further embodiments the compound is selected from the group comprising
- { [3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methoxy}-acetic acid,
  - 25 N-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-succinamic acid,
  - 4-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-butyric acid,
  - { [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methoxy}-acetic acid,
  - 30 4-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-2-phenyl-butyric acid,
  - N-[3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-succinamic acid,
  - 35 2-{ [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methyl}-benzoic acid,

- 2-Chloro-4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(6,8-Dimethyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
5 4-(8-Methoxy-6-methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(6,8-Dimethoxy-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(7,9-Dimethoxy-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
10 4-(6-Methyl-3-oxo-8-trifluoromethyl-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(7,9-Dichloro-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
15 [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-propionic acid,  
[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-acetic acid,  
4-(4-Methyl-3-oxo-3H-chromeno[4,3-c]pyrazol-2-yl)-benzoic  
20 acid,  
4-(3-Oxo-3H-thiochromeno[4,3-c]pyrazol-2-yl)benzoic acid,  
4-(5-Methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoylamino]-3-phenyl-propionic acid, and  
25 2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoylamino]-2-acetic acid.

In yet another embodiment said compound is a CD80 antagonist, capable of inhibiting the interaction between  
30 CD80 and CD28.

The present invention relates in a second aspect to a compound as set forth above for use as a medicament.

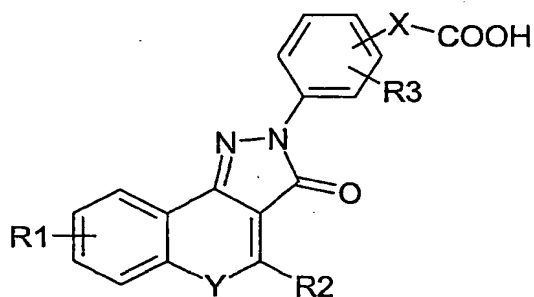
In one preferred embodiment said compound is used as a medicament for treatment of medical conditions chosen  
35 from the group comprising rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis.

The present invention relates in a third aspect to a compound as set forth above for use as a prodrug, preferably in the form of an ester.

The present invention relates in a fourth aspect to a pharmaceutical composition comprising said compound as active ingredient in association with a pharmaceutically acceptable adjuvant, diluent or carrier.

In one preferred embodiment said pharmaceutical composition is used for treatment of medical conditions chosen from the group comprising rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis.

The present invention relates in a fifth aspect to the use of a compound having the general formula (I)



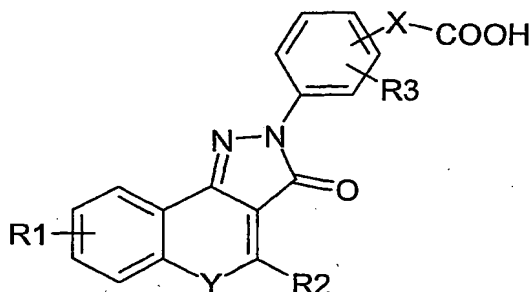
(I)

wherein X represents a bond or a group selected from substituted or unsubstituted C<sub>1-3</sub>-alkyl, NH-C(O)-C<sub>1-3</sub>-alkyl, NH-C(O)-CH<sub>2</sub>-O-CH<sub>2</sub> or C(O)-NH-(amino acid residue);  
Y represents NR<sub>4</sub>, O or S;  
R1 represents H, halo, CF<sub>3</sub>, lower alkyl or lower alkoxy;  
R2 and R4 represents independently H or lower alkyl; and  
R3 represents H, halo, lower alkyl or lower alkoxy,  
wherein halo is F, Cl or Br;  
wherein lower alkyl represents saturated or unsaturated, straight, branched or cyclic alkyl groups having 1-6 carbon atoms; and  
wherein lower alkoxy represents saturated or unsaturated, straight, branched or cyclic alkoxy groups having 1-6

carbon atoms, or pharmaceutically acceptable salts thereof, for the manufacture of a medicament for treatment of medical conditions which benefit from immunomodulation.

5 In one embodiment said medical conditions are chosen from the group comprising rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis.

10 The present invention relates in a sixth aspect to a method for treatment of medical conditions which benefit from immunomodulation comprising administration of a therapeutically effective amount of a compound having the general formula (I)



15

(I)

wherein X represents a bond or a group selected from substituted or unsubstituted  $C_{1-3}$ -alkyl,  $NH-C(O)-C_{1-3}$ -alkyl,  $NH-C(O)-CH_2-O-CH_2$  or  $C(O)-NH-$ (amino acid residue);

20 Y represents  $NR_4$ , O or S;

$R_1$  represents H, halo,  $CF_3$ , lower alkyl or lower alkoxy;

$R_2$  and  $R_4$  represents independently H or lower alkyl; and

$R_3$  represents H, halo, lower alkyl or lower alkoxy,

wherein halo is F, Cl or Br;

25 wherein lower alkyl represents saturated or unsaturated, straight, branched or cyclic alkyl groups having 1-6 carbon atoms; and

wherein lower alkoxy represents saturated or unsaturated, straight, branched or cyclic alkoxy groups having 1-6



carbon atoms, or pharmaceutically acceptable salts thereof.

In one embodiment said medical conditions are chosen from the group comprising rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis.

In another embodiment said therapeutically effective amount per day is within the range of 0,001-10 mg/kg body weight, preferably within the range of 0,1-5 mg/kg body weight.

#### Detailed description of the invention

The present invention relates to heterocyclic compounds, to pharmaceutical compositions comprising them, and to methods and use of said compounds for clinical treatment of medical conditions which may benefit from immunomodulation, e.g. rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis. More particularly the present invention relates to heterocyclic compounds, which are CD80 antagonists capable of inhibiting the interactions between CD80 and CD28.

According to the present invention it has been found that compounds of general formula I are CD80 antagonists. Compounds of the general formula I inhibit the interaction between CD80 and CD28. The CD80 antagonistic properties of the compounds of general formula I have been established in Surface Plasmon Resonance (BIAcore) experiments.

It is preferred that X in the general formula I is a bond and in such cases other substituents, such as an adjacent meta-chloro, improves activity.

The compounds of the present invention may be in the acid form but may also be in the form of pharmaceutically acceptable salts.

The compounds of the present invention may also be in the form of prodrugs, especially esters with appro-

priate alcohols. Prodrugs can have improved pharmacokinetic and/or solubility properties.

Since the compounds of formula I are CD80 antagonists capable of interfering with the CD80 - CD28 interaction they are useful for treatment of inflammatory conditions and autoimmune diseases, e.g. rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis.

Effective quantities of the compounds of formula I are preferably administered to a patient in need of such treatment according to usual routes of administration and formulated in usual pharmaceutical compositions comprising an effective amount of the active ingredient and a suitable pharmaceutically acceptable carrier. Such compositions may take a variety of forms, e.g. solutions, suspensions, emulsions, tablets, capsules and powders prepared for oral administration, sterile solutions for parenteral administration, suppositories for rectal administration or suitable topical formulations. Conventional procedures for the selection and preparation of suitable pharmaceutical formulations are described, for example, in "Pharmaceuticals - The Science of Dosage Form Design", M. B. Aulton, Churchill Livingstone, 1988.

A suitable daily dose for use in the treatment of any of the conditions mentioned above is within the range of 0.001 mg/kg to 10 mg/kg body weight, in particular within the range of 0.01 mg/kg to 5 mg/kg body weight, depending upon the specific condition to be treated, the age and weight of the specific patient, and the specific patient's response to the medication. The exact individual dosage, as well as the daily dosage, will be determined according to standard medical principles under the direction of a physician.

Various additives to enhance the stability or ease the administration of the drug can be added to the formulation. The pharmaceutical composition may also contain

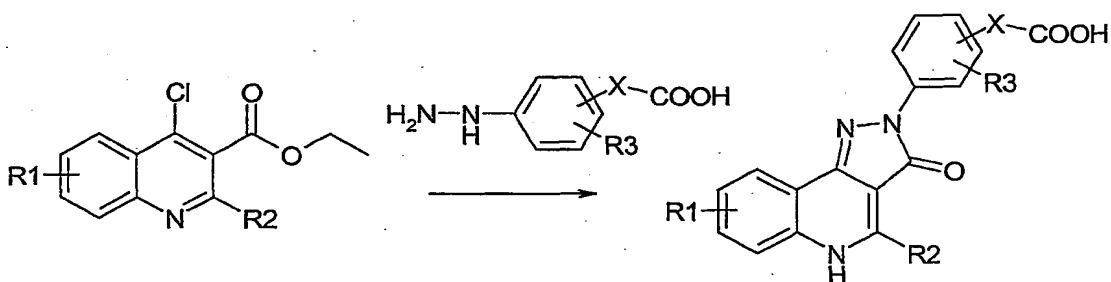
additional therapeutically useful substances other than one or more compounds of the general formula I.

The present invention is further illustrated by the following non-limiting experimental part.

5 Experimental part

The compounds of general formula I may be prepared by the methods described below. The prior art patent documents cited above also include useful synthetic methods.

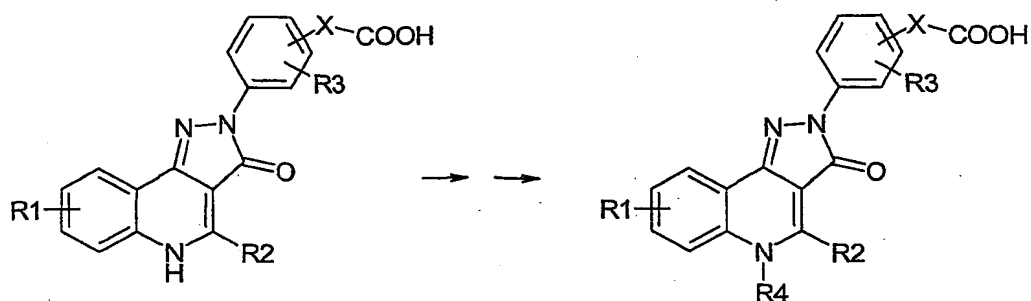
10 Thus, compounds of general formula I wherein Y = N and X is an alkyl chain can be prepared as shown in the reaction below;



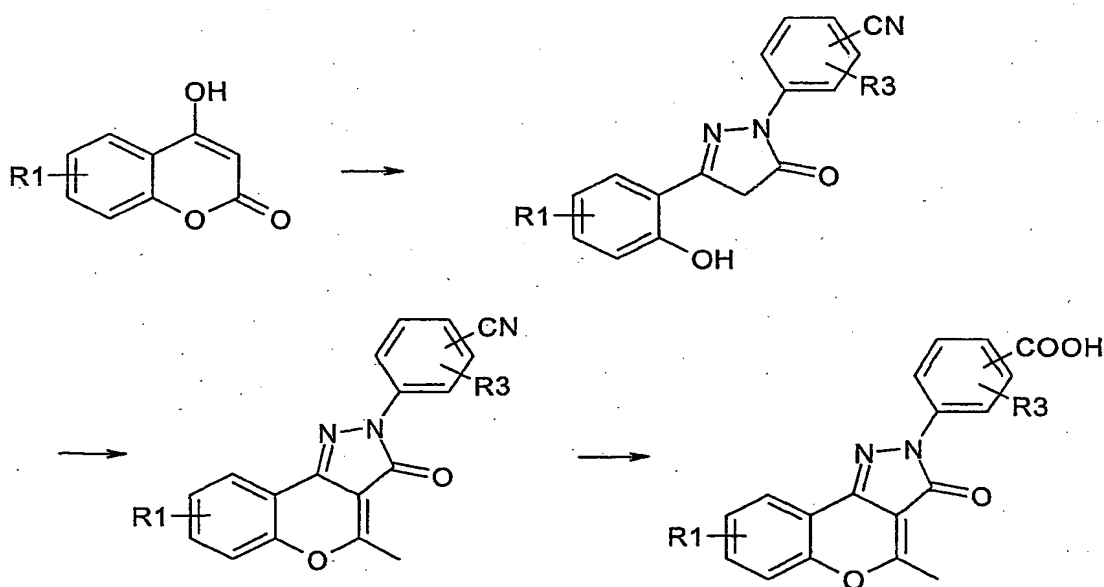
15 The starting material is available by known procedures (e.g. L. Savini et al, Bioorganic & Medicinal Chemistry 6 (1998) 389-399) and the reaction with hydrazine derivatives is performed by heating in a suitable solvent such as n-butanol. In cases where the acid is esterified  
20 under the reaction conditions, hydrolysis gives back the acid. Preparation of hydrazine derivatives was accomplished following literature procedures (Hunsberger et al, J. Org. Chem. 21 (1956) 394, 395, 396. Harden F. A. et al, J. Med. Chem. 34 (1991) 2892-8).

25 Compounds wherein R4 is lower alkyl may be obtained by subsequent alkylation. If the alkylation results in ester formation, the corresponding acid is easily obtained by hydrolysis.

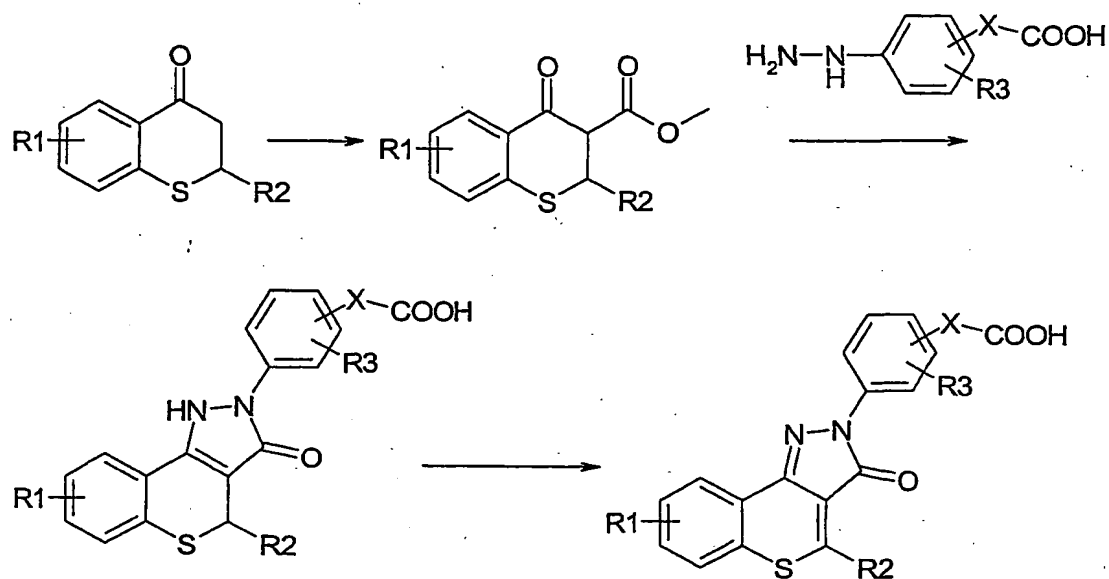
11



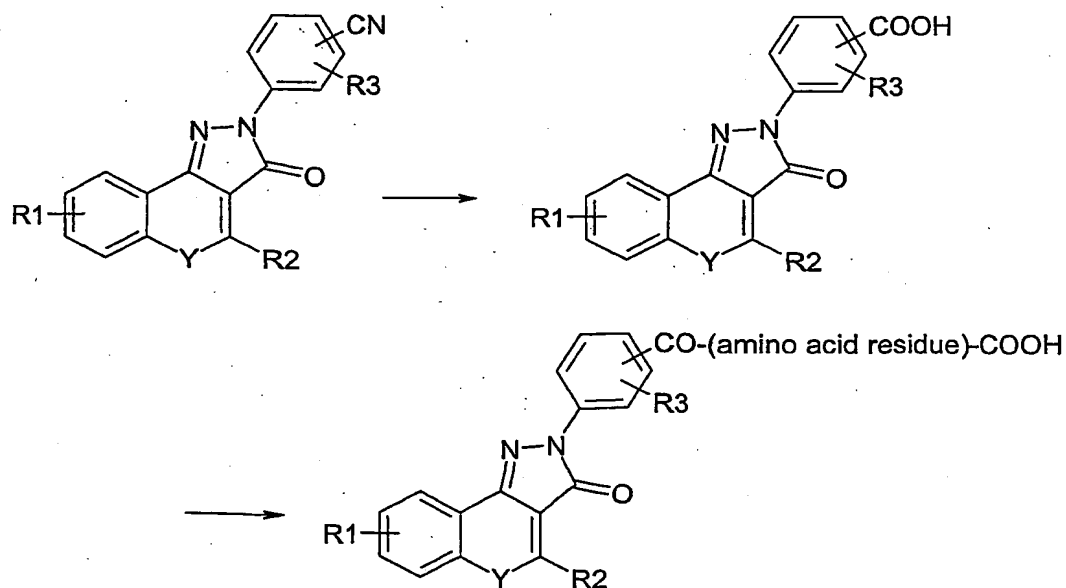
The compounds of general formula I wherein Y = O and X is an alkyl chain can be prepared according to various literature procedures (e.g. Ghosh C. K. Et al, Synthesis 5 (1978) 779-781; Frogett J. A. et al, J. Chem. Research (S) (1997) 30-31). One synthetic route, when R2 = Me, is described below. The cyano group is subsequently hydrolysed to produce the corresponding acid.



10 The compounds of general formula I wherein Y = S and X is an alkyl chain can be prepared as shown below using procedures described in the literature (Donelly M. X. D. et al, J. Chem. Soc. Perkin Trans. 1 (1993) 1729-1735; Lombardino J. G. et al, J. Med. Chem. 24 (1981) 830-834).  
15 The final oxidation was accomplished by stirring in air.

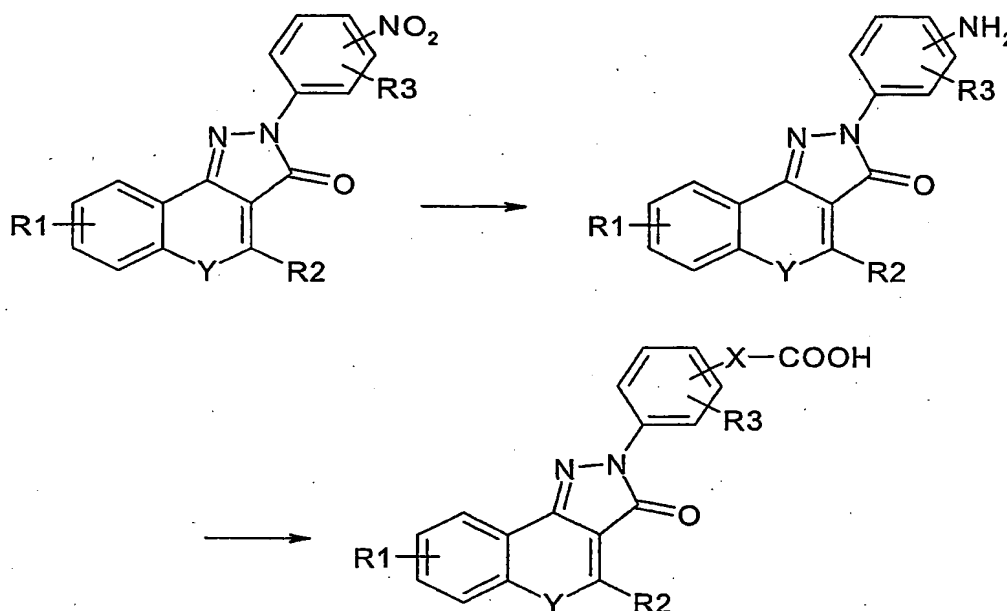


The compounds wherein X is a bond may also be obtained from the corresponding cyanide derivatives and the amino acid derivatives are prepared by condensation of the acid with the appropriate amino acid.



Compounds wherein X is NH-C(O)-(alkyl)- and NH-C(O)-CH<sub>2</sub>-O-CH<sub>2</sub>- can be prepared by reduction of the nitro group (L. Savini et al, Bioorganic & Medicinal Chemistry 6

(1998) 389-399) and subsequent acylation with the appropriate anhydride.



## 5 Examples

The following examples are intended to illustrate the invention without restricting the scope thereof. Compounds were named using Autonom 2.1 from Beilstein. NMR spectra were recorded on a Bruker ARX 400 instrument. Coupling constants in the aromatic area are mostly referred to as singlets (s), doublets (d), triplets (t) in order to reflect the appearance of the NMR spectrum.

### Example 1

{[3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methoxy}-acetic acid

To a suspension of 2-(3-amino-phenyl)-2,5-dihydro-pyrazolo[4,3-c]quinolin-3-one (0.050 g, 0.18 mmol) in DMF (0.8 ml) diglycolic anhydride (0.025 g, 0.22 mmol) and 4-dimethylaminopyridine (0.007 g, 0.05 mmol) were added. The clear solution was stirred at room temperature and the product precipitated during the reaction. After 3 h water was added to the mixture. The precipitate was collected, washed with water and dried to yield {[3-(3-oxo-

3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl-carbamoyl]-methoxy}-acetic acid (0.064 g):  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.8 (1H, bs), 10.02 (1H, s), 8.69 (1H, s), 8.37 (1H, s), 8.18 (1H, d), 7.95 (1H, d), 7.62-7.70 (2H, m), 7.49-7.55 (2H, m), 7.33 (1H, t), 4.19 (2H, s), 4.17 (2H, s); ESI MS  $m/z$  393 ( $\text{M}+\text{H}^+$ )

Using essentially the same procedure the following compounds were prepared:

- 10 *N*-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-succinamic acid  
 $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.98 (1H, s), 8.67 (1H, s), 8.18 (1H, d), 8.08 (2H, d), 7.58-7.70 (4H, m), 7.51 (1H, t), 2.47-2.57 (4H, m); ESI MS  $m/z$  377 ( $\text{M}+\text{H}^+$ ).
- 15 4-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-butyric acid  
 $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  9.89 (1H, s), 8.67 (1H, s), 8.18 (1H, d), 8.08 (2H, d), 7.60-7.69 (4H, m), 7.51 (1H, t), 2.33 (2H, t), 2.25 (2H, t), 1.75-1.83 (2H, m); ESI MS  $m/z$  391 ( $\text{M}+\text{H}^+$ ).
- 20 { [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methoxy}-acetic acid  
 $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  12.76 (1H, bs), 9.86 (1H, s), 8.68 (1H, d), 8.19 (1H, d), 8.11 (2H, d), 7.61-7.69 (4H, m), 7.52 (1H, t), 4.19 (2H, s), 4.15 (2H, s); ESI MS  $m/z$  393 ( $\text{M}+\text{H}^+$ ).
- 25 4-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-2-phenyl-butyric acid  
 $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ ) (contain some 3-phenyl regioisomer)  $\delta$  9.86 (1H, s), 8.67 (1H, s), 8.18 (1H, d), 8.07 (2H, d), 7.58-7.69 (4H, m), 7.51 (1H, t), 7.20-7.38 (5H, m), 3.55 (1H, t), 2.10-2.30 (3H, m), 1.90-2.02 (1H, m); ESI MS  $m/z$  467 ( $\text{M}+\text{H}^+$ ).
- 30 *N*-[3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-succinamic acid  
 $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.05 (1H, s), 8.68 (1H, s), 8.31 (1H, s), 8.17 (1H, d), 7.90 (1H, d), 7.62-7.70 (2H,

m), 7.48-7.55 (2H, m), 7.30 (1H, t), 2.49-2.57 (4H, m);  
ESI MS  $m/z$  377 ( $M+H^+$ ).

*2-{[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methyl}-benzoic acid*

- 5  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  10.11 (1H, s), 8.67 (1H, s), 8.18 (1H, d), 8.08 (2H, d), 7.85 (1H, d), 7.59-7.69 (4H, m), 7.46-7.54 (2H, m), 7.35 (2H, t), 4.07 (2H, s); ESI MS  $m/z$  439 ( $M+H^+$ ).

Example 2

- 10 *4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid*

- A solution of 4-chloro-quinoline-3-carboxylic acid ethyl ester (11.8 mg, 0.5 mmol) and 4-hydrazino-benzoic acid (7.6 mg, 0.5 mmol) in n-butanol (0.5 mL) was stirred  
15 at 115°C over night in a sealed tube. After cooling to 50-70°C, heptane (1.0 mL) was added and the product was allowed to crystallize upon further cooling to room temperature. The solvent was removed and the product was washed with heptane and dried under vacuum to yield 4-(3-  
20 oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid (13 mg).  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.9 (1H, s), 8.77 (1H, d), 8.38 (2H, d), 8.25 (1H, d), 8.03 (2H, d), 7.65-7.77 (2H, m), 7.58 (1H, t).

- Using essentially the same procedure the following  
25 compounds were prepared:

*3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid*

- $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.87 (1H, bs), 8.78 (1H, s), 8.72 (1H, d), 8.46 (1H, d), 8.23 (1H, d), 7.63-7.72 (3H, m), 7.51-7.56 (2H, m); ESI MS  $m/z$  306 ( $M+H^+$ ).

*2-Chloro-4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid*

- In this reaction the hydrochloride salt of 2-chloro-4-hydrazino-benzoic acid was used and therefor  $Et_3N$  (2  
35 eq) was also added to the mixture.  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  13.00 (1H, bs), 8.76 (1H, d), 8.44 (1H, s), 8.22-



8.28 (2H, m), 7.94 (1H, d), 7.65-7.74 (2H, m), 7.55 (1H, t); ESI MS  $m/z$  340 ( $M+H^+$ ).

4-(6-Methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

- 5  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.1 (1H, s), 8.52 (1H, d), 8.38 (2H, d), 8.12 (1H, d), 8.03 (2H, d), 7.56 (1H, d), 7.48 (1H, t), 2.58 (3H, s).

4-(8-Methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

- 10  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.9 (1H, s), 8.73 (1H, d), 8.39 (2H, d), 8.06 (1H, s), 8.03 (2H, d), 7.64 (1H, d), 7.53 (1H, d), 2.50 (3H, s, in DMSO signal).

4-(6,8-Dimethyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

- 15  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.48 (1H, s), 8.38 (2H, d), 8.03 (2H, d), 7.92 (1H, s), 7.40 (1H, s), 2.57 (3H, s), 2.45 (3H, s).

4-(8-tert-Butyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

- 20  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.9 (1H, s), 8.75 (1H, d), 8.40 (2H, d), 8.17 (1H, s), 8.03 (2H, d), 7.81 (1H, d), 7.69 (1H, d), 1.40 (9H, s).

4-(8-Methoxy-6-methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

- 25  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.3 (1H, s), 8.45 (1H, d), 8.41 (2H, d), 8.03 (2H, d), 7.49 (1H, d), 7.21 (1H, d), 3.93 (3H, s), 2.57 (3H, s).

4-(8-Methoxy-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

- 30  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.9 (1H, s), 8.71 (1H, d), 8.41 (2H, d), 8.03 (2H, d), 7.70 (1H, d), 7.61 (1H, d), 7.32 (1H, dd), 3.94 (3H, s).

4-(6,8-Dimethoxy-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

- 35  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.40 (2H, d), 8.31 (1H, s), 8.03 (2H, d), 7.18 (1H, d), 6.93 (1H, d), 4.03 (3H, s), 3.95 (3H, s).

4-(7,9-Dimethoxy-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 12.6 (s, 1H), 8.61 (1H, s), 8.36 (2H, d), 8.01 (2H, d), 6.79 (1H, d), 6.69 (1H, d),  
5 4.00 (3H, s), 3.87 (3H, s).

4-(3-oxo-8-trifluoromethyl-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid (60% pure by NMR)

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 13.1 (1H, s), 8.85 (1H, d), 8.38 (2H, d), 8.10 (1H, s), 8.03 (2H, d), 7.87 (1H, d),  
10 7.73 (1H, d).

4-(6-Fluoro-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 13.0 (1H, s), 8.58 (1H, s), 8.37 (2H, d), 8.06 (1H, d), 8.04 (2H, d), 7.54-7.66 (2H,  
15 m).

4-(6-Chloro-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.47 (1H, s), 8.36 (2H, d), 8.24 (1H, d), 8.04 (2H, d), 7.88 (1H, d), 7.57 (1H, t).

20 4-(8-Chloro-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid (85% pure by NMR)

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 13.0 (1H, s), 8.82 (1H, d), 8.38 (2H, d), 8.20 (1H, s), 8.03 (2H, d), 7.76 (2H, s).

25 4-(7,9-Dichloro-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid (60% pure by NMR)

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 13.0 (s, 1H), 8.81 (1H, s), 8.36 (2H, d), 8.04 (2H, d), 7.78 (1H, d), 7.71 (1H, d).

### Example 3

30 [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-propionic acid

A suspension of 4-chloro-quinoline-3-carboxylic acid ethyl ester (0.050 g, 0.21 mmol) and 3-(4-hydrazino-phenyl)-propionic acid (0.046 g, 0.25 mmol) in 2-propanol (3 ml) was heated at 84°C in an oil bath for 10 h. The  
35 precipitate was collected, washed with 2-propanol and dried to afford the 2-propylester of the product (0.031 g). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 12.8 (1H, bs), 8.66 (1H,

d), 8.18 (1H, d), 8.07 (2H, d), 7.60-7.72 (2H, m), 7.52 (1H, t), 7.26 (2H, d), 4.85 (1H, m), 2.82 (2H, t), 2.55 (2H, t), 1.12 (6H, d).

The ester (0.019 g) was hydrolysed by dissolving it in EtOH (0.8 ml) and 1M NaOH (0.4 ml). After 90 minutes the mixture was acidified with 2 M HCl and the precipitate was collected, washed with water and dried to yield [4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-propionic acid (0.016 g): <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.66 (1H, s), 8.18 (1H, d), 8.07 (2H, d), 8.61-8.69 (2H, m), 7.51 (1H, t), 7.26 (2H, d), 2.81 (2H, t), 2.52 (2H, t); ESI MS m/z 334 (M+H<sup>+</sup>).

Using essentially the same procedure the following compounds were prepared:

15 [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-acetic acid n-butyl ester

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 12.8 (1H, bs), 8.68 (1H, d), 8.18 (1H, d), 8.13 (2H, d), 7.61-7.72 (2H, m), 7.53 (1H, t), 7.30 (2H, d), 4.02 (2H, t), 3.63 (2H, s), 1.52 (2H, m), 1.28 (2H, m), 0.83 (3H, t); and

[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-acetic acid

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.67 (1H, s), 8.19 (1H, d), 8.11 (2H, d), 7.61-7.69 (2H, m), 7.52 (1H, t), 7.29 (2H, d), 3.54 (2H, s); ESI MS m/z 320 (M+H<sup>+</sup>).

#### Example 4

##### 3-(4-Hydrazino-phenyl)-propionic acid

A suspension of 3-(4-aminophenyl)-propionic acid (0.50 g, 3.0 mmol) in concentrated hydrochloric acid (3.5 ml) was treated with sodium nitrite (0.21 g, 3.0 mmol) in H<sub>2</sub>O (1.7 ml) at 0°C. The reaction was stirred for 45 minutes after which stannous chloride (1.26 g, 6.7 mmol) in concentrated hydrochloride acid (1.5 ml) was added dropwise at 0°C. The reaction was stirred for 1 h at room temperature. The precipitate was collected and dried to afford the hydrochloride salt (0.54 g). The salt (0.100 g) was dissolved in a small amount of water and made ba-

sic with 1M NaOH. The solid material was removed by filtration and the filtrate was acidified with acidic acid to yield a precipitate. The solid was collected, washed with water and dried to afford 3-(4-hydrazino-phenyl)-propionic acid (0.050 g):  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  6.90 (2H, d), 6.64 (2H, d), 2.63 (2H, t), 2.39 (2H, t).

Using essentially the same procedure the following compound was prepared:

*2-Chloro-4-hydrazino-benzoic acid hydrochloride*

In this reaction the hydrochloride salt was collected and purified by recrystallization from ethanol:  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.97 (1H, bs), 7.76 (1H, d), 7.03 (1H, s), 6.88 (1H, d).

Example 5

*4-[3-(2-Hydroxy-phenyl)-5-oxo-4,5-dihydro-pyrazolo-1-yl]-benzonitrile*

A suspension of 4-hydroxycoumarine (0.71 g, 4.4 mmol) and 4-cyanophenylhydrazine (0.88 g, 6.6 mmol) in dry toluene was heated at 125°C in an oil bath. Toluene was slowly distilled off during the reaction to separate water. A total of 30 ml toluene was removed. After 3 h the solution was allowed to cool and the precipitate was filtered, washed with toluene and dried. The crude product was dissolved in  $\text{CH}_2\text{Cl}_2$ . The solid material was removed by filtration before the solution was washed with 2M HCl. The organic phase was dried, filtrated and the solvent evaporated to yield 4-[3-(2-hydroxy-phenyl)-5-oxo-4,5-dihydro-pyrazolo-1-yl]-benzonitrile (0.44 g):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.86 (1H, s), 8.01 (2H, d), 7.73 (2H, d), 7.41 (1H, t), 7.23-7.26 (1H, m), 7.08 (1H, d), 6.98 (1H, t), 3.99 (2H, s).

Example 6

*4-(4-Methyl-3-oxo-3H-chromeno[4,3-c]pyrazol-2-yl)-benzonitrile*

A mixture of 4-[3-(2-hydroxy-phenyl)-5-oxo-4,5-dihydro-pyrazolo-1-yl]-benzonitrile (0.25 g, 0.9 mmol) and triethyl orthoacetate (1.6 ml) was heated at 120°C in

an oil bath for 15 minutes. After cooling the precipitate was filtered, washed with diethyl ether and dried to yield 4-(4-methyl-3-oxo-3H-chromeno[4,3-c]pyrazol-2-yl)-benzonitrile (0.22 g): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.35 (2H, d), 8.18 (1H, d), 7.70 (2H, d), 7.58-7.62 (1H, m), 7.47-7.51 (2H, m), 2.84 (3H, s); ESI MS m/z 302 (M+H<sup>+</sup>).

#### Example 7

4-(4-Methyl-3-oxo-3H-chromeno[4,3-c]pyrazol-2-yl)-benzoic acid

A mixture of 4-(4-methyl-3-oxo-3H-chromeno[4,3-c]pyrazol-2-yl)-benzonitrile (0.030 g, 0.10 mmol), acetic acid (0.4 ml), H<sub>2</sub>SO<sub>4</sub> (0.4 ml) and water (0.4 ml) was heated at 100°C in an oil bath for 19 h. After cooling water was added and the precipitation collected, washed with water and dried to yield 4-(4-methyl-3-oxo-3H-chromeno[4,3-c]pyrazol-2-yl)-benzoic acid (0.029 g): <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.21 (2H, d), 8.13 (1H, d), 8.01 (2H, d), 7.67-7.75 (2H, m), 7.54-7.58 (1H, m), 2.78 (3H, s); ESI MS m/z 321 (M+H<sup>+</sup>).

Using essentially the same procedure the following compound was prepared:

4-(3-Oxo-3H-thiochromeno[4,3-c]pyrazol-2-yl)benzoic acid:  
<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 9.29 (1H, s), 8.44 (1H, d), 8.24 (2H, d), 8.03 (2H, d), 7.98 (1H, d), 7.67-7.76 (2H, m); ESI MS m/z 323 (M+H<sup>+</sup>).

#### Example 8

4-Oxo-thiochroman-3-carboxylic acid methyl ester

LHMDS (1.12 g, 6.7 mmol) dissolved in anhydrous THF (7 ml) was cooled to -78°C under N<sub>2</sub>. Thiochroman-4-one (1.00 g, 6.1 mmol) in anhydrous THF (20 ml) was added dropwise under 20 minutes. After an additional 60 minutes methyl cyanoformate (0.62 g, 7.3 mmol) in anhydrous THF (1.5 ml) was added dropwise under 5 minutes and the suspension was then stirred at -78°C for 80 minutes. The suspension was poured onto 10% NH<sub>4</sub>Cl and extracted with ether. The organic phase was washed with water, dried

(Na<sub>2</sub>SO<sub>4</sub>), filtered and the solvent evaporated. The crude product was purified by chromatography on silica gel (heptane-ethyl acetate 10:1) to yield 4-oxo-thiochroman-3-carboxylic acid methyl ester (0.65 g): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>; enol tautomer) δ 12.64 (1H, s), 7.83 (1H, d), 7.25-7.29 (2H, m), 7.16-7.20 (1H, m), 3.84 (3H, s), 3.71 (2H, s).

#### Example 9

4-(3-Oxo-1,4-dihydro-3H-thiochromeno[4,3-c]pyrazol-2-yl)-benzonitrile

4-Oxo-thiochroman-3-carboxylic acid methyl ester (0.200 g, 0.90 mmol), 4-cyanophenylhydrazine (0.132 g, 0.99 mmol) together with a small amount of pivalic acid was heated at 118°C in an oil bath under N<sub>2</sub>. After 1 h the mixture was cooled to room temperature and then triturated with ether. The precipitate was filtered and dried to yield 4-(3-oxo-1,4-dihydro-3H-thiochromeno[4,3-c]pyrazol-2-yl)-benzonitrile (0.233 g): <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.06 (2H, d), 7.92 (2H, d), 7.80-7.83 (1H, m), 7.30-7.33 (1H, m), 7.18-7.25 (2H, m), 3.91 (2H, s).

#### Example 10

4-(3-Oxo-3H-thiochromeno[4,3-c]pyrazol-2-yl)-benzonitrile

A solution of 4-(3-oxo-1,4-dihydro-3H-thiochromeno[4,3-c]pyrazol-2-yl)-benzonitrile (0.100 g, 0.33 mmol) in DMSO (2 ml) was stirred vigorously at room temperature and air was flushed over the solution. After 48 h the precipitation was filtered, washed with toluene and dried. The crude product recrystallized from toluene to yield 4-(3-oxo-3H-thiochromeno[4,3-c]pyrazol-2-yl)-benzonitrile (0.036 g): <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.65 (1H, s), 8.53 (1H, d), 8.38 (2H, d), 7.72 (2H, d), 7.66 (2H, t), 7.58-7.62 (1H, m); ESI MS m/z 304 (M+H<sup>+</sup>).

#### Example 11

4-(5-Methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid

A suspension of 4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid (0.050 g, 0.16 mmol) in DMF

(1 ml) was added in small portions to a suspension of NaH (55%) (0.017 g, 0.39 mmol) in DMF (0.5 ml) under N<sub>2</sub>. The reaction was stirred at room temperature. After 1 h MeI (0.053 g, 0.38 mmol) was added. After additional 24 h water was added and the solid material (mainly consisting of ester product) was removed by filtration. The filtrate was acidified with 2M HCl. The precipitate was collected, washed with water and dried to yield 4-(5-methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid (0.018 g). This material contained approx. 10% of ester product: <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.89 (1H, s), 8.34 (2H, d), 8.29 (1H, d), 7.99 (2H, d), 7.85 (1H, d), 7.76 (1H, t), 7.62 (1H, t), 4.00 (3H, s); ESI MS m/z 320 (M+H<sup>+</sup>).

15 Example 12

*2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoylamino]-3-phenyl-propionic acid*

4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid (30mg, 0.1mmol) was dissolved in DMF (0.5 ml) and 18mg (0.1mmol) of carbonyldiimidazol (90% pure) was added and the mixture stirred for 4 h. Phenylalanine (33mg, 0.2mmol) was dissolved in of H<sub>2</sub>O (0.5 ml) together with triethylamine (0.05 ml) and added to the activated acid. This reaction mixture was heated at 75°C for 2 h. After cooling it was made acidic and diluted 5x with H<sub>2</sub>O. The precipitate was filtered and washed with H<sub>2</sub>O and dried under vacuum. FlashTube chromatography using EtOAc/MeOH(7:3) yielded a small amount of 2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoylamino]-3-phenyl-propionic acid. The material contained approx. 10% of starting material. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.7 (1H, s), 8.31 (2H, d), 8.21 (1H, d), 7.82 (2H, d), 7.70 (1H, d), 7.62 (1H, t), 7.50 (1H, t), 7.2-7.3(4H, m), 7.17 (1H, t), 4.50 (1H, m), 3.1-3.2 (2H,m).

35 Using essentially the same procedure the following compound was prepared:

2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoylamino]-2-acetic acid

<sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.7 (1H, s), 8.36 (2H, d), 8.22 (1H, d), 7.92 (2H, d), 7.70 (1H, d), 7.63 (1H, t),  
5 7.51 (1H, t), 3.85 (2H, d).

Example 13

Time resolved fluorescence competition assay protocol:  
CD28-CD80 interactions.

Reagent	Supplier
Eu-labelled anti-Rabbit antibody (αR-Eu)	Wallac Oy, Turku, Finland,
Rabbit anti-mouse IgG, Fc fragment specific (RαmIg(Fc))	Jackson ImmunoResearch Laboratories Inc.,
Human CD28-mouse IgG1(Fc) (CD28-mFc)	Active Biotech Research AB
Human CD80-mouse C215 Fab (C215Fab-hCD80)	Active Biotech Research AB
Mouse C215Fab	Active Biotech Research AB
Biotin conjugated Goat anti-mouse IgG Kappa light chain (GαMk-biot)	Southern Biotechnology Ass. Inc.
Streptavidin-allophycocyanin (SA-APC)	Wallac Oy, Turku, Finland

10

Assay buffer

50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.8, containing 0.1% BSA (w/v), added prior to use.

Sample preparation

15

The substances to be tested for inhibitory effects were serially diluted from stock concentrations of 20 mM (DMSO) in assay buffer to preparation concentrations of 200, 100, 50 and 25 μM. The final concentration in the wells was 100, 50, 25 and 12.5 μM, respectively. The maximum final DMSO concentration tolerated in the assay was  
20



0.5%. Where appropriate, the dilution series was adjusted and extended to measure the IC<sub>50</sub>.

#### Control

CTLA4-hIg(Fc) was diluted to working concentrations of 20nM and 2nM, resulting in final concentrations of 10nM and 1nM in the well. At these concentrations (10nM and 1nM) approximately 90% and 30% inhibition levels were observed.

#### Preparation of reagent mixture

For 1 plate:

To a tube containing 1.1 ml assay buffer, the following reagents were added:

Reagent	Volume/ 1.1 ml assay buffer	Preparation conc	Final conc in well
αR-Eu	4.2 µl from 0.528 mg/ml	2 µg /ml	1 µg /ml
RαmFc	2.8 µl from 2.4 mg/ml	6 µg /ml	3 µg /ml
CD28-mFc	2.6 µl from 0.4 mg/ml	0.95 µg/ml (10 nM)	0.48 µg/ml (5 nM)
Gαmk-biotin	8.8 µl from 0.5 mg/ml	4 µg/ml	2 µg/ml
SA-APC	15.4 µl from 1 mg/ml	16 µg/ml	8 µg/ml

CD80FabC215 mixture

900 µl of the above reagent mixture was transferred to a new tube. CD80FabC215 was added at a preparation concentration of 20 nM, (i.e. 4.3 µl from 0.4mg/ml stock). The final concentration in the well was 10 nM.

FabC215 mixture

To the remaining 200 µl reagent mixture C215Fab was added as control for non-specific binding (NSB). 0.5 µl from a 0.36 mg/ml stock gave a preparation concentration of 20nM and a final concentration in the well of 10 nM.

*Pipetting*

To a black, half area, 96-well microtiter plate, the C215Fab containing reagent mixture was transferred to column 12, row E-H (4 wells), 10  $\mu$ l/ well with a single channel electronic pipette. 10 $\mu$ l/well of the CD80FabC215 containing reagent mixture was transferred with a single channel electronic pipette to all other wells on the plate

10 B0: Assay buffer, 10  $\mu$ l/well was pipetted to the CD80FabC215 containing wells in column 12, row A-D (4 wells).

NSB: Assay buffer, 10 $\mu$ l/well was pipetted to the C215Fab containing wells in column 12, row E-H (4 wells).

15 CTLA4-hIg(Fc), 10  $\mu$ l/well of 20 and 2nM (final conc 10 and 1nM) was pipetted in duplicate into well 11 E-F and 11 G-H.

Sample dilution series: 10  $\mu$ l/well in duplicate to the remaining wells.

*Incubation*

20 The plate was covered with a plastic lid and incubated in the dark: Initially for 1 h on a shaker platform at room temperature (RTS), stationary at + 4°C overnight, and finally 1 h RTS before reading.

*Measurement*

25 The plate was measured on a Victor 1420 Multilabel Counter using the LANCE protocol (#2) measuring emission at dual wavelengths, from both APC (665nm) and Europium (615nm). First measurement: Excitation 340nm, emission 665nm, delay 50  $\mu$ s, window time 200  $\mu$ s. Second measurement: 30 Excitation 340nm, emission 615nm, delay 50  $\mu$ s, window time 200 $\mu$ s.

*Calculation*

The fluorescence signal ratio 1000\* 665nm/615nm, from which the percentage inhibition was calculated, was 35 determined. A logit b plot (Logit b = LN (%Bound / (100% - % Bound, plotted against Log conc) was performed from which IC<sub>50</sub> was measured.

The assay was performed at least twice in order to have two comparable  $IC_{50}$  values of the compounds.

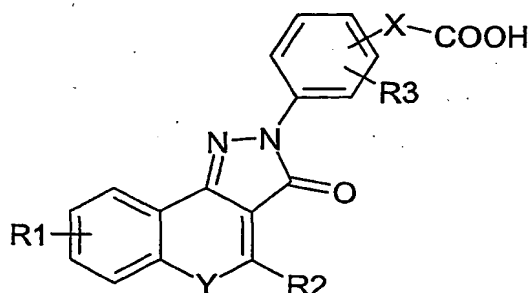
#### Results

The following representative results were obtained:

- 5  
4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-  
benzoic acid  
 $IC_{50} = 0,48 \mu M$
- 10  
2-Chloro-4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-  
yl)-benzoic acid  
 $IC_{50} = 0,27 \mu M$
- 15  
3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-  
benzoic acid  
 $IC_{50} = 0,88 \mu M$
- 20  
N-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-  
phenyl]-succinamic acid  
 $IC_{50} = 0,6 \mu M$
- 25  
[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-  
phenyl]-propionic acid  
 $IC_{50} = 1,6 \mu M$
- 30  
2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-  
benzoylamino]-2-acetic acid  
 $IC_{50} = 3,9 \mu M$
- 35  
4-(3-Oxo-3H-thiochromeno[4,3-c]pyrazol-2-yl)benzoic acid  
 $IC_{50} = 13,5 \mu M$

## CLAIMS

1. A compound having the general formula (I)



(I)

wherein X represents a bond or a group selected from substituted or unsubstituted C<sub>1-3</sub>-alkyl, NH-C(O)-C<sub>1-3</sub>-alkyl, NH-C(O)-CH<sub>2</sub>-O-CH<sub>2</sub> or C(O)-NH- (amino acid residue);  
 10 Y represents NR<sub>4</sub>, O or S;  
 R<sub>1</sub> represents H, halo, CF<sub>3</sub>, lower alkyl or lower alkoxy;  
 R<sub>2</sub> and R<sub>4</sub> represents independently H or lower alkyl; and  
 R<sub>3</sub> represents H, halo, lower alkyl or lower alkoxy,  
 wherein halo is F, Cl or Br;  
 15 wherein lower alkyl represents saturated or unsaturated, straight, branched or cyclic alkyl groups having 1-6 carbon atoms; and  
 wherein lower alkoxy represents saturated or unsaturated, straight, branched or cyclic alkoxy groups having 1-6  
 20 carbon atoms,  
 with the proviso that R<sub>2</sub> is not H, when X is a bond, Y is NH and R<sub>3</sub> is H,  
 or pharmaceutically acceptable salts thereof.

2. A compound according to claim 1, wherein X is a  
 25 bond.

3. A compound according to claim 1 or 2, wherein Y is NH.

4. A compound according to any one of claims 1-3, selected from the group comprising

- { [3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methoxy}-acetic acid,  
N-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-succinamic acid,  
5 4-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-butyric acid,  
{ [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methoxy}-acetic acid,  
4-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-2-phenyl-butyric acid,  
10 N-[3-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-succinamic acid,  
2-{ [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenylcarbamoyl]-methyl}-benzoic acid,  
15 2-Chloro-4-(3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(6,8-Dimethyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(8-Methoxy-6-methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
20 c]quinolin-2-yl)-benzoic acid,  
4-(6,8-Dimethoxy-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(7,9-Dimethoxy-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
25 4-(6-Methyl-3-oxo-8-trifluoromethyl-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
4-(7,9-Dichloro-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,  
[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-propionic acid,  
30 [4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-phenyl]-acetic acid,  
4-(4-Methyl-3-oxo-3H-chromeno[4,3-c]pyrazol-2-yl)-benzoic acid,  
35 4-(3-Oxo-3H-thiochromeno[4,3-c]pyrazol-2-yl)benzoic acid,  
4-(5-Methyl-3-oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoic acid,

2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoylamino]-3-phenyl-propionic acid, and  
2-[4-(3-Oxo-3,5-dihydro-pyrazolo[4,3-c]quinolin-2-yl)-benzoylamino]-2-acetic acid.

5           5. A compound according to any one of claims 1-4, which is a CD80 antagonist, capable of inhibiting the interaction between CD80 and CD28.

6. A compound according to any one of claims 1-5 for use as a medicament.

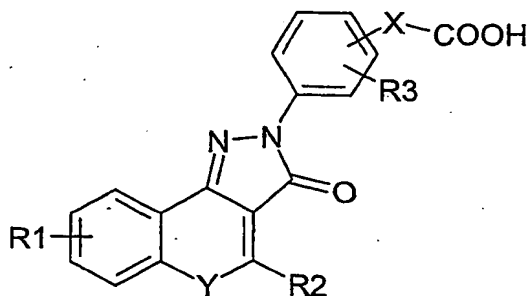
10           7. A compound according to claim 6 for use as a medicament for treatment of medical conditions chosen from the group comprising rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and psoriasis.

15           8. Use of a compound as defined in any one of claims 1-5 as a prodrug, preferably in the form of an ester.

9. A pharmaceutical composition comprising a compound according to any one of claims 1-6 as active ingredient in association with a pharmaceutically acceptable  
20           adjuvant, diluent or carrier.

10. A pharmaceutical composition according to claim 9 for treatment of medical conditions chosen from the group comprising rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus  
25           erythematosus and psoriasis.

11. Use of a compound having the general formula (I)



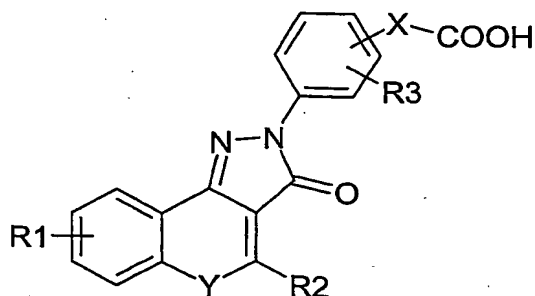
(I)

wherein X represents a bond or a group selected from substituted or unsubstituted C<sub>1-3</sub>-alkyl, NH-C(O)-C<sub>1-3</sub>-alkyl, NH-C(O)-CH<sub>2</sub>-O-CH<sub>2</sub> or C(O)-NH- (amino acid residue); Y represents NR<sub>4</sub>, O or S;

- 5 R<sub>1</sub> represents H, halo, CF<sub>3</sub>, lower alkyl or lower alkoxy;  
R<sub>2</sub> and R<sub>4</sub> represents independently H or lower alkyl; and  
R<sub>3</sub> represents H, halo, lower alkyl or lower alkoxy,  
wherein halo is F, Cl or Br;  
wherein lower alkyl represents saturated or unsaturated,  
10 straight, branched or cyclic alkyl groups having 1-6  
carbon atoms; and  
wherein lower alkoxy represents saturated or unsaturated,  
straight, branched or cyclic alkoxy groups having 1-6  
carbon atoms, or pharmaceutically acceptable salts there-  
15 of, for the manufacture of a medicament for treatment of  
medical conditions which benefit from immunomodulation.

12. Use according to claim 11, wherein said medical  
conditions are chosen from the group comprising rheuma-  
toid arthritis, multiple sclerosis, diabetes, asthma,  
20 transplantation, systemic lupus erythematosus and pso-  
riasis.

13. A method for treatment of medical conditions  
which benefit from immunomodulation comprising admini-  
stration of a therapeutically effective amount of a com-  
25 pound having the general formula (I)



(I)

wherein X represents a bond or a group selected from substituted or unsubstituted C<sub>1-3</sub>-alkyl, NH-C(O)-C<sub>1-3</sub>-alkyl, NH-C(O)-CH<sub>2</sub>-O-CH<sub>2</sub> or C(O)-NH- (amino acid residue); Y represents NR<sub>4</sub>, O or S;

- 5 R<sub>1</sub> represents H, halo, CF<sub>3</sub>, lower alkyl or lower alkoxy; R<sub>2</sub> and R<sub>4</sub> represents independently H or lower alkyl; and R<sub>3</sub> represents H, halo, lower alkyl or lower alkoxy, wherein halo is F, Cl or Br; wherein lower alkyl represents saturated or unsaturated,  
10 straight, branched or cyclic alkyl groups having 1-6 carbon atoms; and wherein lower alkoxy represents saturated or unsaturated, straight, branched or cyclic alkoxy groups having 1-6 carbon atoms, or pharmaceutically acceptable salts  
15 thereof.

14. A method according to claim 13, wherein said medical conditions are chosen from the group comprising rheumatoid arthritis, multiple sclerosis, diabetes, asthma, transplantation, systemic lupus erythematosus and  
20 psoriasis.

15. A method according to claim 13 or 14, wherein said therapeutically effective amount per day is within the range of 0,001-10 mg/kg body weight, preferably within the range of 0,1-5 mg/kg body weight.



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 02/01309

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C07D 471/04, C07D 491/052, C07D 495/04, A61K 31/4745, A61K 31/4162,  
A61P 3/10, A61P 11/06, A61P 17/06, 19/02, 37/02  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07D, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CHEM. ABS DATA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0354693 A1 (THE BOOTS COMPANY PLC), 14 February 1990 (14.02.90), see claims and page 7, line 10 - line 17 --	1-15
X	EP 0354694 A1 (THE BOOTS COMPANY PLC), 14 February 1990 (14.02.90), see claims and page 7, line 2 - line 9 --	1-15
X	WO 9111448 A1 (THE BOOTS COMPANY PLC), 8 August 1991 (08.08.91), see claims and page 29, line 10 - line 24 --	1-15

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

6 November 2002

07-11-2002

Name and mailing address of the ISA/  
Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Nebil Gecer/EÖ  
Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 02/01309

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4268516 A (JOSEPH G. LOMBARDINO ET AL), 19 May 1981 (19.05.81), see claims and e.g. column 6, line 20 - line 29 --	1-15
X	WO 9106298 A1 (PHARMACIA AB), 16 May 1991 (16.05.91), see claims --	1-15
X	WO 9734893 A1 (ASTRA PHARMACEUTICALS LTD.), 25 Sept 1997 (25.09.97), see claims and page 15, line 12 - line 35 --	1-15
A	Bioorganic & Medicinal Chemistry, Vol. 6, 1998, L. Savini et al: "High Affinity Central Benzodiazepine Receptor Ligands: Synthesis and Structure-Activity Relationship Studies of a New Series of Pyrazolo(4,3-c)quinolin-3-ones", page 389 - page 399, see table 1, page 391-392 --	1-15
A	US 4312870 A (NAOKATA YOKOYAMA), 26 January 1982 (26.01.82) -- -----	1-15

## INTERNATIONAL SEARCH REPORT

International application No.  
**PCT/SE02/01309****Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **13-15**  
because they relate to subject matter not required to be searched by this Authority, namely:  
**see next sheet**
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/SE02/01309

Claims 13-15 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

30/09/02

International application No.

PCT/SE 02/01309

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
EP	0354693	A1	14/02/90	AU	4060289	A	05/03/90
				CN	1069271	A	24/02/93
				DD	284019	A	31/10/90
				DK	88790	A	09/04/90
				FI	901753	D	00/00/00
				HU	58337	A	28/02/92
				HU	208977	B	28/02/94
				HU	894659	D	00/00/00
				JP	3500418	T	31/01/91
				MX	17041	A	28/02/94
				NO	901573	A	06/06/90
				NZ	230231	A	26/03/92
				PH	26314	A	29/04/94
				PT	91408	A	08/03/90
				WO	9001481	A	22/02/90
				YU	155189	A	28/02/91
				ZA	8905646	A	25/04/90
				GB	8818894	D	00/00/00
EP	0354694	A1	14/02/90	AU	625915	B	16/07/92
				AU	4042689	A	05/03/90
				DD	287039	A	14/02/91
				DK	85290	A	05/04/90
				FI	901754	D	00/00/00
				GB	8818895	D	00/00/00
				HU	58743	A	30/03/92
				HU	205763	B	29/06/92
				HU	894573	D	00/00/00
				JP	3500417	T	31/01/91
				MX	17042	A	31/01/94
				NO	901574	A	06/06/90
				NZ	230232	A	25/10/91
				PH	26639	A	04/09/92
				PT	91409	A	08/03/90
				WO	9001482	A	22/02/90
				YU	155289	A	31/10/91
				ZA	8905645	A	25/04/90

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

30/09/02

International application No.

PCT/SE 02/01309

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
WO	9111448	A1	08/08/91	AU	7220991 A	21/08/91
				BR	9105984 A	10/11/92
				CA	2074841 A	03/08/91
				CS	9100238 A	15/09/91
				EP	0539372 A	05/05/93
				FI	923486 A	31/07/92
				GB	9002314 D	00/00/00
				HU	9202510 D	00/00/00
				IE	910365 A	14/08/91
				IL	97114 D	00/00/00
				IN	171735 A	26/12/92
				JP	5505180 T	05/08/93
				MX	24401 A	01/10/93
				NO	923037 A	30/09/92
				PT	96656 A	31/10/91
				ZA	9100764 A	30/10/91
				GB	9002315 D	00/00/00
				GB	9002425 D	00/00/00
-----						
US	4268516	A	19/05/81	NONE		
-----						
WO	9106298	A1	16/05/91	SE	8903564 D	00/00/00
-----						

## INTERNATIONAL SEARCH REPORT

Information on patent family members

30/09/02

International application No.

PCT/SE 02/01309

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9734893 A1	25/09/97	AU 712141 B	28/10/99
		AU 2186797 A	10/10/97
		BR 9708103 A	27/07/99
		CA 2247814 A	25/09/97
		CN 1218472 A	02/06/99
		CZ 9802977 A	17/03/99
		EE 9800298 A	15/02/99
		EP 0888347 A	07/01/99
		GB 9605803 D	00/00/00
		IL 126271 D	00/00/00
		JP 2000506884 T	06/06/00
		NO 984290 A	27/10/98
		NZ 331614 A	28/07/00
		PL 328921 A	01/03/99
		SK 118798 A	10/03/99
		TR 9801861 T	00/00/00
		ZA 9702150 A	22/09/97
		GB 9610474 D	00/00/00
		GB 9610894 D	00/00/00
		AU 719553 B	11/05/00
		AU 2170397 A	22/10/97
		BR 9708478 A	13/04/99
		DE 69709075 D,T	22/08/02
		DK 890013 T	08/04/02
		EP 0890013 A,B	13/01/99
		SE 0890013 T3	
		GB 9700862 D	00/00/00
		NO 984548 A	27/11/98
		US 6170578 B	09/01/01

INTERNATIONAL SEARCH REPORT  
Information on patent family members

30/09/02

International application No.

PCT/SE 02/01309

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US	4312870	A	26/01/82	AT 8629 T 15/08/84
			AU 538890 B 30/08/84	
			AU 5945580 A 08/01/81	
			CA 1148159 A 14/06/83	
			CY 1357 A 07/08/87	
			DD 151754 A 04/11/81	
			DE 3068673 D 00/00/00	
			DK 160762 B,C 15/04/91	
			DK 264880 A 22/12/80	
			EP 0022078 A,B 07/01/81	
			SE 0022078 T3	
			FI 68827 B,C 31/07/85	
			FI 801964 A 22/12/80	
			GR 69282 A 13/05/82	
			HK 47387 A 26/06/87	
			HU 183156 B 28/04/84	
			IE 49993 B 22/01/86	
			IE 801279 L 21/12/80	
			IL 60357 A 31/05/84	
			JP 1650145 C 30/03/92	
			JP 3009114 B 07/02/91	
			JP 56018980 A 23/02/81	
			KR 8400421 B 02/04/84	
			MY 55687 A 31/12/87	
			NO 153430 B,C 09/12/85	
			NO 801866 A 22/12/80	
			NZ 194102 A 12/04/83	
			PT 71420 A 01/07/80	
			SG 14387 G 10/07/87	
			ZA 8003714 A 24/06/81	